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Original Article

# Characterizing the Water Immersion Finger Wrinkling Test in Healthy Subjects

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## Abstract

Water immersion skin wrinkling (WISW) test is a simple and can be used to assess sympathetic sudomotor function. Our objective was to determine the association between WISW and other sympathetic tests like cold pressor test (CPT), Maximum Voluntary Contraction (MVC) and Head Up Tilt (HUT) in healthy adults. The participants were subjected to WISW, HUT, MVC and CPT. Wrinkling scores were obtained prior to water immersion, 5, 15 and 30 minutes post immersion. A positive correlation ($r=0.32$, $p<0.01$) was obtained between WISW scores and change in DBP during HUT. An inverse association (–0.50, $p<0.01$) between WISW and age was also observed suggesting that WISW is sensitive to subtle physiological changes such as changes in age. Reproducibility of the test was tested for by two different observers and the means were same. Maximal wrinkling was at 15 minutes of immersion suggesting that this the optimal duration for wrinkling.

## Introduction

Autonomic neuropathy affects nerves that control involuntary activities of the body (1, 2). Autonomic neuropathy can present with symptoms that occur when there is damage to the nerves supplying vital organs of the body (3) and could involve alteration in regulation of blood pressure, heart rate, bowel and bladder emptying and digestion. Cardiovascular system involvement in autonomic neuropathy has been demonstrated to be associated with fatal complications (4). For instance reports of cardiac arrhythmias, cardiac failure or even myocardial infarction have been linked to autonomic neuropathy. Our own data published recently on type 2 diabetic Indians have demonstrated autonomic neuropathy much earlier than development of peripheral neuropathy (3). Further, data on type 2 diabetics have demonstrated that early detection of autonomic dysfunction helps control complications associated with it (3, 5). All pointing towards early detection and treatment of autonomic dysfunction. Though the complications associated with autonomic neuropathy could be debilitating, evaluation still remains a distant process. The reasons for this could be manifold such as lack of well-equipped autonomic laboratories or of technical expertise or lack of knowledge to detect early changes in autonomic nervous system. Whatever might be the reason autonomic nervous

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system evaluation remains an area least explored.

Various techniques have been proposed to evaluate autonomic dysfunction. Using beat to beat heart rate (HR) and blood pressure (BP), tests have been devised to evaluate cardiac sympathetic or parasympathetic nervous system (3, 6). In fact, we have been able demonstrate that even standard 12 lead ECG could be used as an effective tool to diagnose autonomic dysfunction in a peripheral set up (6, 7). However, the same cannot be said about the sympathetic vasomotor or sudomotor evaluation.

Sympathetic sudomotor function is said to be involved much earlier than any other part of autonomic nervous system including cardiac sympathetic or parasympathetic fibers (3, 8). Available techniques such as sympathetic skin response, Q-sart, sweat imprint are difficult to perform, requires expertise and specialized equipment hence limiting their application in the clinical settings. Hence, arises a need for a test which is simple, easy to perform and reliable, to detect sudomotor component of the autonomic nervous system.

There have been reports suggesting water immersion finger wrinkling testis simple (9, 10) and non invasive (11) which can be employed to evaluate peripheral sympathetic functioning (12, 13). Water immersion finger wrinkling is a dynamic process (14) occurring as a result of peripheral vasoconstriction of the digital arteries (15, 16) when fingers are immersed in warm water the temperature of which is 40°C. One of the most important functions of sympathetic system in the limbs is maintenance of vascular tone. Vasoconstriction occurring in the digital vessels brings about wrinkling in the digits and hence water immersion finger wrinkling tests the peripheral sympathetic function. Abnormal results during finger wrinkling test has most often been reported in various diseased states. Finger wrinkling is diminished in conditions causing autonomic neuropathies (10, 17, 18) such as parkinsonism (19), diabetes (18, 19). It is also decreased following sympathectomy (17), in replanted fingers after amputation (20, 21), leprosy (13, 22), acute phase of Guillian-Barre syndrome (22), small fiber neuropathies (9), familial dysautonomia (19) and congestive cardiac failure (23).

However prior to advocating the use of WISW test as a bedside clinical test it is important to maximally characterize the WISW test. Our study is an attempt to describe the WISW test in terms of optimal duration required for water immersion, inter-observer variability on the outcome measures, different methods to assess the wrinkling induced by water immersion and the effect of age and gender on the rate of formation of water immersion induced wrinkles. We also attempted to compare WISW test with other sympathetic tests namely Head Up Tilt (HUT), Sustained Isometric Contraction (SIC) and Cold Pressor Test (CPT).

## Materials and Methods

Sixty two healthy subjects 27 males and 35 females in the age group of 18-50 years were recruited from in and around the medical college over a period of 21 months from September 2011 to May 2013. Sample size was calculated assuming a correlation coefficient of 0.4 obtained from our own pilot study with an alpha error of 5% and power of 80% the final sample size calculated was 60. Subjects with clinical history of autonomic dysfunction, diabetes, hypertension, peripheral vascular disease, previous hand surgery or significant trauma in the past were excluded from the study. Subjects who were pregnant and lactating were not included in the study.

#### Anthropometry

All subjects underwent detailed anthropometry which included both measured parameters including height to the nearest 0.1 cm (Holtain Ltd., Crymych, Britain), weight to the near 0.1 Kg (Tanita; Tokyo, Japan), skin folds thickness (Lange skinfold caliper; Cambridge, Maryland), and mid arm circumference.

#### Sympathetic function tests

All subjects underwent the sympathetic function tests in random order. Cold pressor test was done at the end. Following each test a rest period of one minute was given.

**Head up tilt :**

The subject was made to lie supine on the tilt table. After obtaining a baseline blood pressure and heart rate the subject was tilted upright to an angle of 70°(24). The tilt was for a period of 5 minutes (24). BP and HR were recorded using oscillometric method at baseline, immediately after tilting, end of 3 minutes and 5 minutes. For calculating the delta (Ä) changes in systolic BP (SBP) and diastolic BP (DBP) difference between basal and minimum values were considered. For calculating the delta changes in heart rate (HR) difference between basal and maximum values were considered.

**Maximum Voluntary Contraction :**

Subject was instructed to apply force on to the handgrip dynamometer to produce a force that is 30% of the individual's maximum voluntary contraction. The subject was asked to sustain the effort for period of 3 minutes. BP and HR were recorded at baseline and at the end of 3 minutes (24). Delta changes in SBP, DBP and HR at the end of 3 minutes were computed.

**Cold pressor test :**

Subject was asked to immerse one hand in ice-cold water 4°C for 90 seconds while heart rate and blood pressure was recorded (24). Delta changes in SBP, DBP and HR were determined. For calculating the delta changes in SBP, DBP and HR basal and maximum values were considered for both sustained isometric contraction and cold pressor tests.

**Water immersion finger wrinkling test :**

Participants were instructed not to use any cream or lotion on their hands on the day of testing (15). They were asked to avoid drinking coffee or tea 2 hours prior to testing (15). During the finger wrinkling test the right hand was immersed in a bucket filled with water at 40°C (15). Temperature was maintained at 40°C over the entire test period. Maximum decrease in temperature by 2°C was allowed over the total measurement period of 30 min (15). The number of wrinkles was observed by two different observers at baseline, 5, 15 and 30 min of immersion. Photographs of the wrinkles were taken at baseline, 5 minutes, 15 minutes and 30 minutes of water immersion finger wrinkling using. Fingerprints of the digits at baseline and post immersion were obtained on a smoked paper and imprints of the wrinkles four digits excluding the thumb were counted as an attempt of better end point quantification of wrinkles.

**Statistics**

The data was normally distributed and results is represented as Mean±SD. Pearson's correlation coefficient was used to represent the associations between WISW scores with other parameters. Repeated measures ANOVA was used to test the mean WISW scores by different observers. A paired T test was used to compare the mean wrinkling scores obtained at different duration of immersion. A Bland Altman plot was used to compare two methods of quantification of wrinkles. The results were considered significant at a p value <0.05.

## Results

The demographic and anthropometric characters of the study population are summarized in Table I. The results are expressed as mean and standard deviation. The study population included both healthy males (n=27) and females (n=35) with the mean age of 27.8 years and BMI of 22.8 Kg/m$^2$. The formation of wrinkles diminished significantly as the age advanced as shown in Graph 1. Table II shows the association between various tests and WISW. Significant positive correlation was obtained between WISW scores and change in diastolic blood pressure during head up tilt. Graph 2 shows that 15 minutes

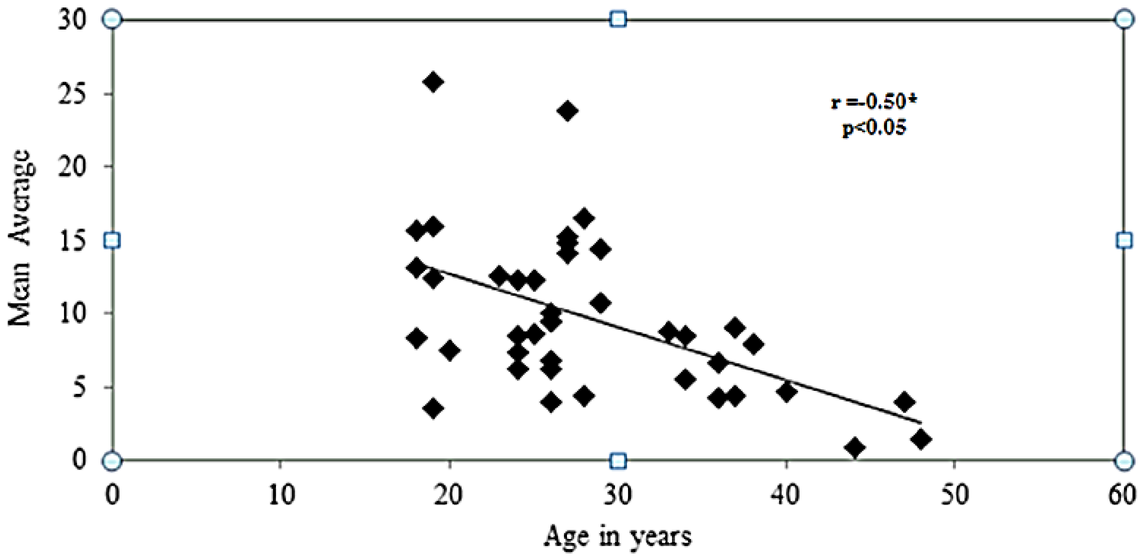
TABLE I : Baseline characters of the study population.

| *Parameters (n=62)* | *Mean±SD* |
|---|---|
| Age (years) | 27.8±7.8 |
| Height (m) | 1.6±0.08 |
| Weight (Kg) | 59.3±9.7 |
| BMI (Kg/m$^2$) | 22.8±3.4 |
| %Fat | 28.5±.5 |
| Fat mass (Kg) | 17.2±4.7 |
| Fat free mass (Kg) | 42.3±6.1 |

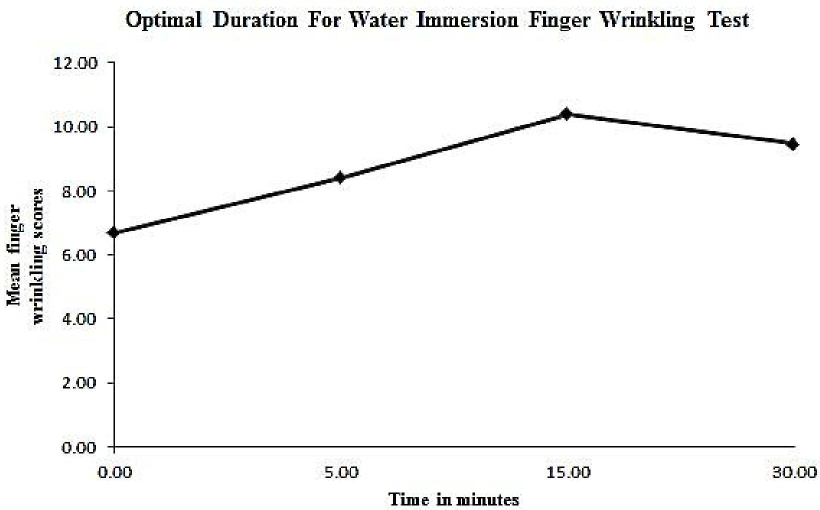
TABLE II : Shows the association between various sympathetic function tests and WISW scores.

| | *Average Wrinkling* | *HUT Δ SBP* | *HUT Δ DBP* | *HUT Δ HR* | *MVC Δ SBP* | *MVC Δ DBP* | *MVC Δ HR* | *CP Δ SBP* | *CP Δ DBP* | *CP Δ HR* |
|---|---|---|---|---|---|---|---|---|---|---|
| Average Wrinkling | 1 | | | | | | | | | |
| HUT Δ SBP | 0.127 | 1 | | | | | | | | |
| HUT Δ DBP | 0.363** | .542** | 1 | | | | | | | |
| HUT Δ HR | –0.106 | 0.002 | .287* | 1 | | | | | | |
| MVC Δ SBP | –0.018 | –0.154 | –0.036 | –0.160 | 1 | | | | | |
| MVC Δ DBP | 0.164 | –0.208 | –0.021 | –0.206 | .676** | 1 | | | | |
| MVC Δ HR | 0.057 | 0.171 | 0.061 | –.292* | 0.172 | .272* | 1 | | | |
| CP Δ SBP | –0.192 | –0.182 | –0.152 | 0.013 | 0.094 | –0.010 | 0.002 | 1 | | |
| CP Δ DBP | –0.012 | 0.019 | 0.006 | –0.154 | 0.226 | 0.105 | 0.270 | .648** | 1 | |
| CP Δ HR | –0.009 | 0.182 | 0.130 | –0.014 | 0.046 | 0.180 | 0.148 | –0.164 | –0.002 | 1 |

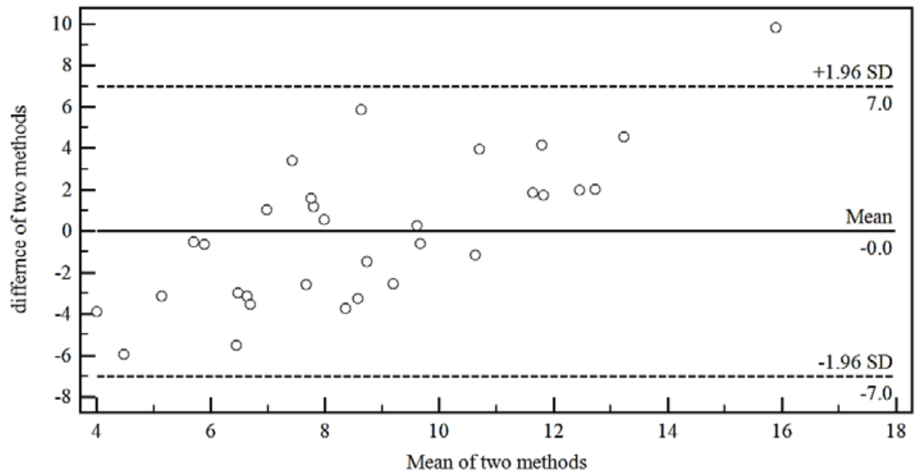
HUT – Head Up Tilt; MVC – Maximum Voluntary Contraction; CP – Cold Pressor Test "SBP – change in Systolic BP; "DBP – change in Diastolic BP; "HR – change in Heart Rate. *significance at a level p<0.05.




Graph 1 : Represents the inverse association of age with mean wrinkling scores on water immersion.




Graph 2 : Represents optimal duration required for water immersion finger wrinkling test *means are significantly different from mean at 0 minute (p<0.01). #means are significantly different from mean at 15 minutes (p<0.01).




Graph 3 : Is a bland altmann plot showing the agreement between two methods of end point quantification of water immersion skin wrinkling namely manual counting vs. counting of wrinkles from finger prints obtained on smoked paper.

was the duration at which wrinkling was maximal and it was significantly higher than the scores at the end of 30 minutes. A Bland Altman plot was used to compare two methods of end point assessment namely visual inspection of wrinkles and finger print method. Finger print method showed a systematic bias when compared with visual inspection method.

## Discussion

In our study we were able to show that WISW test is a sensitive indicator of sympathetic function. An inverse negative association of finger wrinkles with age suggests, that the test can be used to detect even subtle changes in sympathetic functioning. It is known that with advancing age there is a decrease in sympathetic activity (25-27). Similarly, the delta diastolic blood pressure and heart rate change during maximum voluntary contraction showed a significant inverse association with age.

Maintenance of blood pressure during change of posture is an important function of sympathetic nervous system and is mediated by arterial baroreflex (28). There was a significant positive correlation which was observed between the water immersion skin wrinkling scores and the delta change in the diastolic blood pressure during head up tilt. This is similar to the findings by van Barneveld et al and suggests that Finger wrinkling is an indicator of sympathetic functioning. However we found no significant association between results of cold pressor test and maximum voluntary contraction. This could probably be due to the difference in afferent and efferent pathways of these reflexes. We suggest that finger wrinkling test could provide additional information on sympathetic nervous system functioning and should be used along with the other conventional sympathetic tests. van Barneveld et al based on their pilot study suggested that wrinkled fingers could probably predict the outcome of the tilt table test and more the basal wrinkling, poorer would be the outcome. In our study we found a significant positive correlation between the wrinkling scores at baseline prior to water immersion and the delta change in the diastolic blood pressure. This indicates more the basal wrinkling better would be the outcome of the HUT testing. This is in contrast to the study done by van Barneveld et al.

Inter-observer variability of WISW test was done in our study for 21 subjects by two observers. The mean wrinkling scores of the two observers was found to be same .This indicates that the test has little inter-observer variation and counting of the wrinkles is not merely subjective. These results are similar to the other studies (9-11).

In the present study the mean wrinkling scores at 5 minutes, 15 minutes and 30 minutes were significantly different from the mean prior to immersion. In our study the minimum duration required to get a response was 5 minutes and the duration of immersion at which maximal wrinkling was obtained was at 15 minutes after which there was a significant decline in the mean scores. The decrease in the number of wrinkles observed at 30 minutes is probably not due to the decrement in the rate of wrinkle formation but due to the difficulty in estimation of wrinkles as the wrinkles begin to fuse with each other forming loops (9). This result indicates that the optimal duration for water immersion finger wrinkling would be 15 minutes and the minimum time required to get a response would be 5 minutes. In most studies the duration of immersion considered is anywhere between 20 to 30 minutes (10, 15, 18-20). Some studies have used a total immersion time of 12 minutes (11).

A significant positive correlation was observed between the two methods of wrinkling estimation namely manual counting vs. counting of wrinkles from finger prints. Hence a Bland Altmannplot was used. The mean difference between both the methods was +6. The observations between the two methods were lying within ±2SD but a systematic bias was observed with the finger print method underestimating at lesser wrinkling scores and overestimating at higher wrinkling scores.

**Conclusion**

The optimal duration required for water immersion finger wrinkling test according to our study is 15 minutes. The test was also found to be reliable as the wrinkling scores by the observers were not different. In our study we also found an inverse association with age and this suggests that WISW is sensitive to subtle physiological changes such as change in age. WISW scores and change in diastolic blood pressure during head up tilt has significant positive correlation. Visual inspection of wrinkles still remains better than finger print method.

**Limitations :**

The diurnal variation in the finger wrinkling was not tested for, this would be important as there are circadian variations observed in autonomic nervous system functioning (29). It is known that tonicity of the fluid affects the rate of wrinkle formation [30, 31], but it is unknown whether the tonicity of the fluid in our study differed for each subject.

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